AD____________________

Award Number: W81XWH-06-1-0034

TITLE: Prostate Cancer in African-American Men: Serum Biomarkers for Early Detection Using Nanoparticles

PRINCIPAL INVESTIGATOR: Catherine M. Phelan, M.D., Ph.D.

CONTRACTING ORGANIZATION: Moffitt Cancer Center,
Tampa, Florida, 33612-9497

REPORT DATE: November 2009

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

☒ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 0704-0188*

1. REPORT DATE *01-11-2009*	**2. REPORT TYPE** FINAL	**3. DATES COVERED** *1 NOV 2005 - 31 OCT 2009*

4. TITLE AND SUBTITLE
Prostate Cancer in African-American Men: Serum Biomarkers for

Early Detection Using Nanoparticles

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0034

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Catherine M. Phelan, MD, PhD (PI)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Moffitt Cancer Center

Tampa, FL 33612-9497

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Medical Research and
Fort Detrick,
Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT We have collected blood samples from 65 African-American men with prostate cancer and 80 ethnically-matched control healthy men with questionnaire data on demographics, general health and cancer family history. We chose six biomarkers PSA, KLK2, KLK14, IL6, CAV-1, anti-p53 antibody and OPG as candidates for improved early detection of prostate cancer in plasma from the African American men with and without prostate cancer. We had technical problems with KLK2 and KLK14. Furthermore despite their biological pausability, anti-p53 levels were higher in controls than cases and OPG and CAV-1 showed similiar levels in cases and controls. Therefore only PSA was shown to be a suitable consistent biomarker. The use of nanoscale materials and devices has enhanced the lower limits of detection of proteins and other compounds in plasma and tissues. We have investigated using nanoparticles or quantum dots conjugated to proteins of interest for early cancer detection. Although sub-ELISA levels of proteins were detected, this technology is somewhat cost-prohibitive and not very robust. So we developed a gold nanowire biosensor surface with electrochemical detection on which the PSA antibody is immobilized, We proved the efficiency and sensitivity of this new biosensor for early prostate cancer detection. This study has laid the groundwork to make an impact in early prostate cancer detection through essential investigation into emerging cutting-edge technologies. In the future, the utilization of the gold nanowire microfluidic devices will impact early prostate cancer detection with more appropriate biomarkers.

15. SUBJECT TERMS
African-American, prostate cancer, biomarkers

16. SECURITY CLASSIFICATION OF: **U**			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	25	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

A. Introduction

In this study we set up a prostate cancer case-control study in order to develop new serum biomarkers for early detection of prostate cancer using Nanotechnology. In early prostate cancer when there are very few cells, we expect trace serum levels of proteins. Recent advances in nanotechnology mean that target biomarkers of prostate cancer can be conjugated to quantum dots, thereby enhancing detection characteristics and making it possible to detect very low levels of a particular protein (1-9). We collected blood samples from 65 African-American men with prostate cancer and 90 ethnically-matched control healthy men with questionnaire data on demographics, general health and cancer family history to investigate prostate cancer risk factors. Our Aims were to investigate emerging nanotechnologies for their use in early detection of prostate cancer in African American men and to test six biologically plausible candidate biomarkers. We have investigated the QD-conjugated serum biomarkers in a multiplex panel. However, we have found this technology to be cost-prohibitive when moving from the test samples to a larger sample population. We tried to dilute the QD-AB so as to reduce costs but the results are inconsistent and non-reproducible. We tested six antibodies as potential biomarkers at the end of this project we have determined that the most suitable biomarker is still PSA. The most optimal and cost-effective nanotechnology was to conjugate the PSA antibody onto gold nanowire encased microfluidic devices which can detect sub-ELISA levels of PSA. This project is essential in terms of expanding research on the African American populations and vital to improve early detection of prostate cancer so as to reduce the dismal mortality rate. However, additional biomarkers must be identified to enhance specificity and sensitivity of early detection of prostate cancer. This study has shown that the technology is available for enhanced detection. This technology will translate rapidly to other cancer sites for which no good screening test is available.

B. Body

Task 1. To collect pre-treatment serum samples from a cohort of African-American undergoing screening for prostate cancer (Months 1-26 according to the Timeline in the Statement of Work)

Progress:

1.1. Recruitment

Approach: In this study prostate cancer cases are defined as African American men with biopsy-proven prostate cancer. Controls are African American men with a PSA< 2.5ng/ml and no other evidence of prostate cancer by DRE or biopsy. Our case-control collection was slow but we have collected 65 African-American men with prostate cancer and 90 African-American controls into this study. Once consent was obtained, the participants were asked to fill in a short research questionnaire and two blood samples (10 ml each) were drawn. Recruitment took place at Lifetime Cancer Screening at the H. Lee Moffitt Cancer Center and Research Institute and Moffitt Cancer Center Hospital, the Radiation Oncology clinics in Tampa and Brandon and the 30th Street Clinic. All samples were transferred to the Tissue Procurement and the LCS laboratory at the H. Lee Moffitt Cancer Center and Research Institute and processed for serum and DNA, respectively, where they are stored. Original consents and questionnaires are stored in a locked cabinet in a locked office. The questionnaire data collected is scanned into an Access database by the Survey Methods Core. The risk factor database will be linked with the laboratory database, both are in Access.

1.1. Summary: Task 1: Recruitment was slow and we reached to 65 prostate cancer cases and 90 controls in the timeframe of the award. Completed.

Task 2. To engineer a panel of quantum dot (QD) – antibody (AB) conjugates targeting established prostate cancer biomarkers (antigens). (Months 2-10 in the Timeline in the Statement of Work).

Approach: The experimental luminescent QDs will be composed of surface passivated II-VI semi-conductors such as ZnS, CdS, ZnSe, CdSe, or CdTe and their core/shell structures, e.g. CdSe/ZnS, CdTe/CdS, or CdS/ZnS. We will target QDs to cover emission in the broad visible to infrared spectral range to achieve optimal spectral performance. The QDs will be directly conjugated to antibodies to the selected prostate cancer biomarkers: Prostate Specific Antigen (PSA), Kallikrein 2 (KLK2), Kallikrein 14 (KLK14), Osteoprotegerin (OPG), Antip53Ab, Caveolin-1 (Cav-1) and Interleukin-6 (IL-6), with or without linker molecules, depending on the molecular structure of each antibody.

We purchased antibodies (multiple times) for the above biomarkers. Previously, standard curves were determined for each antigen/antibody using ELISA kits (CanAg, CA, USA). The lower limit of detection for this technique was approximately

<0.1ug/l. Therefore we determined that the antibodies were detecting the chosen biomarker. We have successfully conjugated antibodies to the above bio markers to quantum dots and we have gone ahead and are characterizing them according to Task 3 below.

<u>2.1. Summary</u>: Task 2 completed

Task 3. To define the photoluminescence signatures of bound versus unbound QDs created in Aim 2, reflecting antigen-antibody complex formation. **(Months 11-25 according to the Statement of Work)**
Approach: High-resolution photoluminescence (PL) spectroscopy will be used to analyze antibodies and QDs separately before and then after they have been conjugated. This permits the identification of unique spectral signatures, and is based on standard PL descriptors such as: PL peak position at 400nm – 1,700nm spectral range, full width at half maximum (FWHM) of the PL band, optical/acoustic phonon frequencies, excitation wavelength dependence (different laser sources), light polarization, temperature quenching, and PL quantum efficiency. From the PL descriptors of the optimized AB-QD conjugates we will generate a calibration curve of the PL intensity versus concentration of the conjugated QDs in serum/plasma. This allows determination of the sensitivity limit of the QD luminescence diagnostics, limits of concentration linearity and dynamic range.

3.1. Progress: Our goal was to identify a sensitive and specific panel of biomarkers employing reproducible and robust technology at a reasonable cost so it can be applied as a screening test to the general population. We extensively investigated the chemistry of bound versus unbound QDs in ten ways: 1. ELISA; 2. Different types of QDs; 3. Same AB, different QDs; 4. Same QD, different ABs; 5.Storage time; 6. Temperature; 7. Spectral mapping; 8. Gel electrophoresis; and 9. 'Home-made' QDs;

We published a paper on the application of the bio-conjugated quantum dots (QDs) for "sandwich" enzyme linked immunosorbent assay (ELISA). QD-ELISA can detect PSA antigen at concentrations as low as 0.01ng/ml which is ~50 times lower than the classic "sandwich" ELISA was demonstrated. Scanning photoluminescence (PL) spectroscopy was performed on dried ELISA wells and the results compared with the same QD samples dried on a solid substrate. We confirmed a "blue" 37 nm PL spectral shift in a case of QDs conjugated to PSA antibodies. Increasing of the "blue" spectral shift was observed at lower PSA antigen concentrations. The results can be used to improve sensitivity of "sandwich" ELISA cancer antigen detection. Currently, the threshold of PSA AG detection for "sandwich"-ELISA with organic dyes, but for many ELISA kits the threshold is 1 ng/ml [1-4], which is usually low enough for early cancer detection, but "sandwich" ELISA with QDs could possibly detect as low as 0.01 ng/ml of PSA AG. One of the current problems in QD usage for biomedical applications is that bioconjugation reactions may be incomplete and result in residual non-conjugated QDs in the same bio-conjugated solution. Our recent experiments demonstrated that PL spectra of QDs are changed by bioconjugation [5-9]. This is manifested as a blue or short-wavelength spectral shift of the PL maximum, which can be clearly observed as a color change of the dried bioconjugated sample. This unique spectroscopic feature of the bioconjugated QDs may serve as a fingerprint of the bioconjugation reaction. Ultimately, this will dramatically improve sensitivity of biomolecules detection using QDs because a background PL from the non-conjugated QDs can be spectrally separated. In this paper, we report on PL spectroscopic study of the dried "sandwich" ELISA wells, utilizing QDs to detect PSA AG at the concentration range of 0.01 – 1.0 ng/ml. The agarose gel electrophoresis technique with the organic dye fluorescamine is employed to assure the quality of the conjugate, used for "sandwich"-ELISA technique. A "blue" spectral shift magnitude was found to be larger for ELISA "sandwiches" in comparison to the same bio-conjugated QDs, dried on a flat silicon substrate. This effect can be attributed to the elevated stress applied to the individual QD, involved into the "sandwich" formation, as well as more or less efficient, but probably not complete, nonconjugated QD elimination caused by the washing procedure. The PL signal from the bio-conjugated QDs was found in all wells, containing AG, with different concentration but was undetectable in the control well without AG. It is expected that the results can provide strong benefits for early cancer detection and forensic technology.

3. 2. Conclusions "Sandwich" ELISA bimolecular tool enhanced with conjugated 705nm QDs instead of commonly used luminescent dyes was employed to detect a PSA AG in the concentration range from 0.01 to 1.0 ng/ml. Three ELISA wells with AG concentrations 1.0, 0.1 and 0.01 ng/ml shows the PL peak, originated from the conjugated QDs in the sandwich structure with corresponding AG (PSA). The control well without AG molecules shows a negligible QD PL

intensity. A short-wavelength, blue PL spectral shift was observed on all ELISA wells, utilizing conjugated QDs, and the values of this spectral shift (13-37 nm) were enhanced in comparison to the same QDs, conjugated to PSA AB, dried on silicon substrate (2-26nm). This fact was attributed to effective washing of nonconjugated QDs from the ELISA wells, which are always present in a conjugate mixture and therefore contribute to an average PL of the dried QD samples. In addition, a negative correlation was observed between an average "blue" PL spectral position and AG concentration. The origin of this effect is under investigation and presumably attributed to decreasing of elastic stress at higher AG concentrtaions. If confirmed, this will benefit the ultralow limits of detection for biomolecules, because "blue" spectral shift will increase with AG concentration decreasing, making it easier to distinguish between PL coming from "sandwiches" and possible traces of nonconjugated QDs or other contamination at ultralow concentrations of a target molecule. This effect will also be useful in predicting the unknown biomolecule concentration based on the spectral shift position alone, eliminating a need for PL intensity assessment. The results of this research could lead to critical improvements for *in vitro* cancer antigen detection sensitivity using the QD luminescent tagging technique.

However, the cost of QDs and antibodies together is approx. $800. From this two conjugations can be performed. This results in only a small volume of conjugated QD-AB and we can assay only about six samples. We performed experiments with different dilutions of the QD-AB conjugate of 1:10, 1:4, and 1:2 to determine if the QD-AB solution could be diluted but would still reveal detection of the proteins. However, the results were very inconsistent. The 1 in 10 dilution did not work at all (results not shown).

Therefore we looked to other nanotechnologies.

3.3. Utilization of additional nanotechnologies: In the last annual report we revealed our interest in exploring two additional nanotechnologies. Microfluidic devices and Surface enhanced Raman scattering (Zhang Lab.). We have proceeded with the former technology. We have included two very experienced and well published researchers Dr. Shekhar Bhansali who also previously integrated PSA into a microfluidic device for prostate (and other) cancer detection [10-16] and Dr. Weihong Tan who is already performing the Cell-SELEX in prostate cancer and has identified aptamers to prostate cancer cell surface proteins. We have been awarded funds to perform research with Dr. Tan. However, in this study we focused on the utilization of microfluidic devices. This is within the scope of the Specific Aims in this proposal and we modified the Statement of Work to reflect this new direction.

3.4. Testing of candidate biomarkers: We had chosen six candidate biomarkers based on the literature (PSA, KLK2, KLK14, OPG, anti-P53 antibodies, IL6 and CAV-1). We tested these biomarkers for their ease of conjugation to nanoparticles and nanowires as well as their effectiveness as biomarkers for early prostate cancer detection in plasma from the African American men using classical ELISA as well as QD-associated ELISA.
KLK2 and KLK14 antibodies proved technically difficult to conjugate and were not available in and ELISA kit so we excluded them from the analysis.
Anti-P53 antibodies were easy to conjugate but unfortunately were identified at higher levels in controls compared to cases (see results for P53 below). The most suitable biomarker would be one which is increasing in concentration as the stage of cancer increase and increases compare to control levels.
OPG, IL6 and CAV-1 showed very similar levels in the plasma from cases and controls so cut-off points could not be determined (see results for OPG below).

#	PCa status	PSA, ng/ml	P53, U/ml	OPG, pg/ml
1	+	**2.4**	**n/d**	**756**
2	+	**2.6**	**n/d**	**958**
4	+	**3.93**	**n/d**	**1141**
5	+	**4.4**	**n/d**	**842**
7	-	1.6	n/d	840
8	-	<0.5 (n/d)	n/d	699
9	+	**5.93**	**4.1**	**483**

10	+	**2.63**	**15.4**	**951**
12	+	**0.8**	**n/d**	**771**
13	+	**40.67**	**n/d**	**1002**
14	+	**4.13**	**n/d**	**1116**
15	+	**1.47**	**n/d**	**2484**
16	+	**4.27**	**5.2**	**822**
17	+	**4.8**	**n/d**	**876**
18	+	**5.27**	**n/d**	**1050**
20	+	**4.47**	**n/d**	**1926**
21	+	**14.27**	**n/d**	**1095**
22	-	3.53	n/d	972
23	-	4.4	n/d	1491
24	-	0.6	n/d	987
25	+	**2.27**	**n/d**	**1299**
26	+	**12.87**	**n/d**	**1020**
27	+	**4.4**	**4.8**	**720**
28	+	**14.8**	**3.7**	**1524**
29	-	<0.5 (n/d)	8	558
30	+	**3.8**	**n/d**	**630**
31	+	**2.8**	**n/d**	**717**
33	-	2.27	n/d	1254
34	-	0.47	n/d	417
35	+	**1.4**	**n/d**	**669**
36	-	1.8	n/d	882
37	+	**3.87**	**3.6**	**612**
38	+	**12.1**	**n/d**	**1170**
39	-	2.93	n/d	849
42	+	**2.0**	**n/d**	**864**
43	-	0.87	n/d	942
45	+	**2.92**	**n/d**	**943**
46	-	<0.5 (n/d)	n/d	876
48	-	0.9	n/d	861
49	-	1.13	141.7	633
50	-	0.67	4.2	804
51	-	0.80	n/d	1074
52	-	0.53	35.7	879
53	-	N/A	n/d	492
54	-	<0.5 (n/d)	n/d	2001
55	-	<0.5 (n/d)	n/d	612
56	-	1.07	n/d	384
57	-	<0.5	n/d	789
58	-	<0.5	4.1	846
59	-	1.4	n/d	915
60	-	<0.5 (n/d)	n/d	936
61	-	1.13	N/A	N/A
62	-	0.67	4.6	648
68	+	**2.93**	**5.7**	**714**
81	+	**4.67**	**5.1**	**678**
83	-	5.27	n/d	1116
85	+	**4.27**	**n/d**	**705**
86	-	<0.5 (n/d)	n/d	930
87	-	<0.5 (n/d)	7.8	804
88	-	0.6	3.6	489
90	-	<0.5 (n/d)	N/A	N/A
91	-	3.1	5.7	N/A

	PSA	P53	OPG
Total tested	**61**	**60**	**59**
Cases +	29	28	29
Controls -	32	32	30
Expression detected (ED) total	**52 (85%)**	**15 (25%)**	**59 (100%)**
ED cases	29 (100%)	8 (29%)	29 (100%)
ED controls	24 (75%)	7 (22%)	30 (100%)
Average cases	**6.1 ng/ml**	**6.12 u/ml**	**0.984 ng/ml**
Average controls	**1.1 ng/ml**	**25.99 u/ml**	**0.866 ng/ml**

29 cases and 33 controls, 62 total

P53: 60 samples tested, 15 have detectable p53 expression, including 8 cases and 7 controls. The average expression for cases is 6.12 u/ml, and 25.99 u/ml for controls.

OPG: 59 samples tested, 59 (all) have detectable p53 expression. The average expression for cases (29) is 984 pg/ml, and 866 pg/ml for controls (30).

PSA: 61 samples tested, 9 have no detectable PSA (<0.5 ng/ml), all controls. Average PSA for cases (29) is 6.1 ng/ml, and 1.1 ng/ml for controls (32), including those with undetectable PSA levels.

From the Access database based on clinically derived PSA VALUES:

		case=1 or control=0	
		0	**1**
PSA value	**Median**	0.90	4.98
	Q1	0.60	4.10
	Q3	1.50	7.60
Family history of Prostate cancer			
No	**N**	65	32
yes	**N**	6	7

3.5. To develop microfluidic device biosensors: The chosen antibodies will be immobilized onto a gold nanowire biosensor surface with electrochemical detection. Upon protein binding, changes in the electrical properties in the vicinity of an electrode are detected.

Electrochemical sensors is a new and promising group of sensing devices, allowing increased sensitivities in conjunction with lower cost, low analysis times, miniaturized and affordable platforms [17-24]. There are numerous detection techniques, currently being researched towards an application in electrochemical sensors, which are described in detail elsewhere [25-34]. Among them, Electrochemical impedance spectroscopy (EIS) is a powerful and sensitive technique to characterize surface-modified electrodes and for the investigation of electrochemical systems and processes [35-37].

In this paper, we report on a successful fabrication of an impedance-based miniaturized biosensor (Imp-sens) and its application for an ultrasensitive PSA detection in standard and real human plasma solution, spiked with different PSA concentrations. The Imp-sens exhibited the sensitivity of 1 pg /ml for PSA which is at least 10 times lower than any most

sensitive commercial ELISA on the market. An average detection time with Imp-sens for one sample is ~45 minutes, compared to at least 2.5 hours with a standard ELISA. Successful approach to minimize the non-specific binding (NSB) issue has been attempted.
The Imp-sens novel technique may open an era of research geared towards ultransensitive, inexpensive and convenient testing procedures. The results may be useful for anyone, interested in the field of electrochemical portable sensing research.

3.5.1. Chemicals and reagents

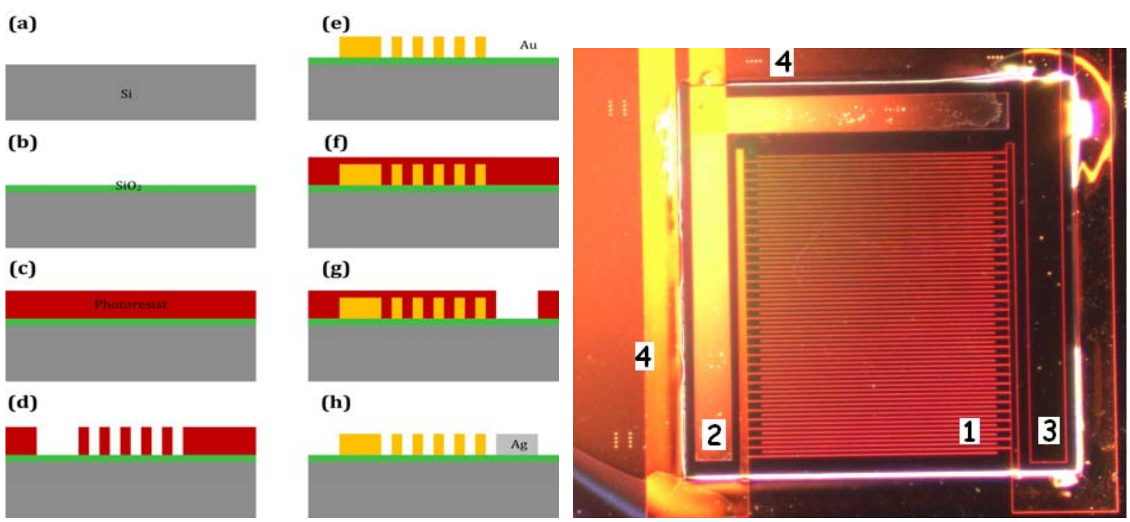
Dithiobis(succinimidyl propionate) (DTSP) and sodium borohydride ($NaBH_4$) were purchased from ThermoFisher Scientific. Purified PSA protein (product # 7820-0604), Monoclonal PSA antibody (anti-PSA, Mab, product # 7820-0217) and monoclonal cortisol antibody (product # 2330-4839) were procured from Abd serotec. Phosphate buffered saline (PBS) tablets were purchased from Sigma Aldrich. SU-8 resist was purchased from Microchem Corp. All other chemicals were of analytical grade and were used without further purification. PBS solution (10 mM, pH 7.4) was prepared by dissolving 1 tablet in 200 ml of deionized water. Working solutions of both the PSA, anti-PSA and anti-cortisol were prepared by dilution in PBS (10 mM, pH 7.4).

3.5.2. Measurement and apparatus

Electrochemical impedance spectroscopy (EIS) was utilized to characterize the -EA/Mab/DTSP/IDµE bio-electrodes and to estimate PSA concentration. EIS measurements were carried out at equilibrium potential without external biasing in the frequency range of 0.5–10^5 Hz with a 5 mV amplitude using Autolab Potentiostat/Galvanostat (Eco Chemie, Netherlands). EIS measurements were carried out using 65 µl of PBS solution (10 mM, pH 7.4) containing a mixture of 5 mM $Fe(CN)_6^{4-}$ (Ferrocyanide) and 5 mM of $Fe(CN)_6^{3-}$ (Ferricyanide) i.e. 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe. Using the redox probe (5 mM $Fe(CN)_6^{3-/4-}$), change in charge transfer resistance (R_{ct}) at electrode/electrolyte interface has been investigated in electrochemical impedance.

3.5.3. Test chip fabrication

The Imp-sens chips were fabricated on an oxidized 4” silicon wafer using standard photolithography techniques [36]. Briefly, Cr/Au (200/2000 Å) layers deposited using e-beam evaporation and were patterned through liftoff. IDµE with 5µm wide electrode fingers at a pitch of 10 µm were used. As a final step, SU 8 chamber patterned around the electrodes using the SU8 50 to create a sample well around these electrodes (Fig1A). Further SU8 was hard baked at 200 ^{o}C to improve its resistance against hard solvents like acetone. Figure 1B reveals the actual photograph of an Imp-sens reagent chamber under optical microscope (10X).



A B

Figure 1. A: Imp-sens fabrication process flow: (a) RCA clean Si wafer, (b) thermal oxidation to grow 500 nm SiO_2 as an insulation layer, (c) apply photoresist, (d) expose and pattern photoresist, (d) deposit Au using electron beam (e-beam) evaporator with thickness of 200 nm and then lift-off photoresist to get pattern Au electrodes, (f) apply photoresist, (g) pattern photoresist, and (h) finally despite Ag using e-beam evaporator with thickness of 300 nm and lift-off is used to remove photoresist and metal on top of it.

B: Imp-sens actual view under the microscope (10X): 1. gold working interdigitated electrodes; 2. silver reference electrode; 3. gold counter electrode; 4. the SU8 reagent chamber.

3.5.4. Self-assembled monolayer (SAM) preparation and antibody immobilization

The Imp-sens chips were pre-cleaned with acetone, isopropyl alcohol, and de-ionized water, and exposed to 2 mg ml^{-1} solution of DTSP in acetone for 1hr for SAM formation. DTSP solution was first reduced using $NaBH_4$ and then dispensed on the pre-cleaned chips at room temperature. The DTSP SAM modified electrodes were then rinsed with acetone to remove any unbound DTSP followed by water rinsing and utilized for antibody immobilization. PSA antibodies were covalently attached to DTSP self-assembled monolayer by incubating the electrode in 65 µl of 1 µg ml^{-1} antibody in PBS solution (10 mM, pH 7.4), for 1 hr. Covalent binding (amide bond formation) results from the facile reaction between amino group of antibody and reactive succinimidyl group of the DTSP on the SAM surface. The sensor (Mab/DTSP/IDµE) was washed thoroughly with PBS (10 mM, pH 7.4) to remove any unbound biomolecules followed by a 10 min washing with ethanolamine (EA) (1%) EA was used to block unreacted succinimidyl group on DTSP SAM and to remove extra unbound antibodies onto the electrode surface. Figure 2 schematically illustrates (a) IDµE chip, (b) Schematic illustration of step-by-step immunochemical reaction on the electrode surfaces, and (c) Proposed detection system. The fabricated bio-electrodes were characterized using the electrochemical impedance technique. Each sample was run in triplicates to ensure repeatability, and an average result of three runs was used.

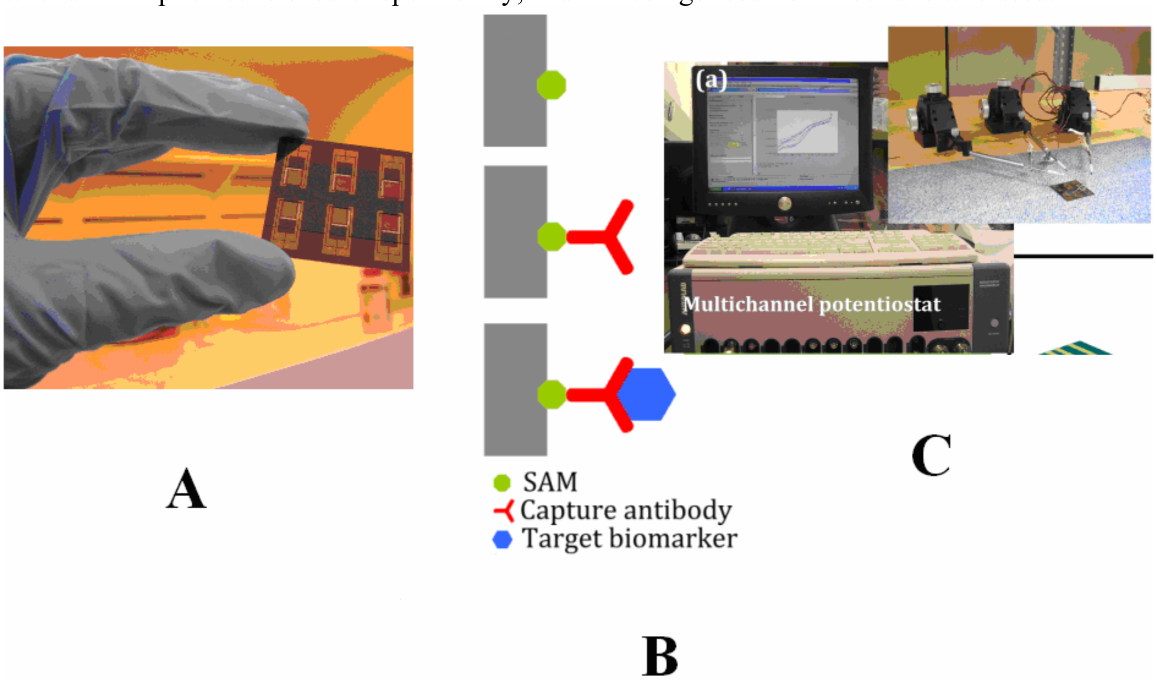


Figure 2. Imp-sens. A: Testing chambers with patterned microelectrodes on Si substrate (6 reagent chambers incorporated into one platform); B: Brief schematic illustration of step-by-step immunochemical reaction on the electrode surfaces; C: Proposed detection system. The multichannel potentiostat is used to scan each reaction chamber on Imp-sens.

3.5.5. Electrochemical impedance studies

Electrochemical impedance spectroscopy (EIS) is a powerful and sensitive technique to characterize surface-modified electrodes and for the investigation of electrochemical systems and processes [35, 38]. It uses periodic small amplitude AC signal over wide frequency range to measure resistive and capacitive response of materials [39-40]. EIS respond to change caused by binding of analyte to bio-recognition elements immobilized on the surface of the electrodes. Nyquist plots of impedance spectra in present studies have been exploited to study (i) change in charge transfer resistance

(R_{ct}) at sensor-solution interfaces after DTSP SAM formation, Mab binding and EA blocking, (ii) the change of charge resistance with changing concentration of PSA. All EIS spectra were recorded in PBS (10 mM, pH 7.4) containing 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe.

3.5.6. Sample collection and storage

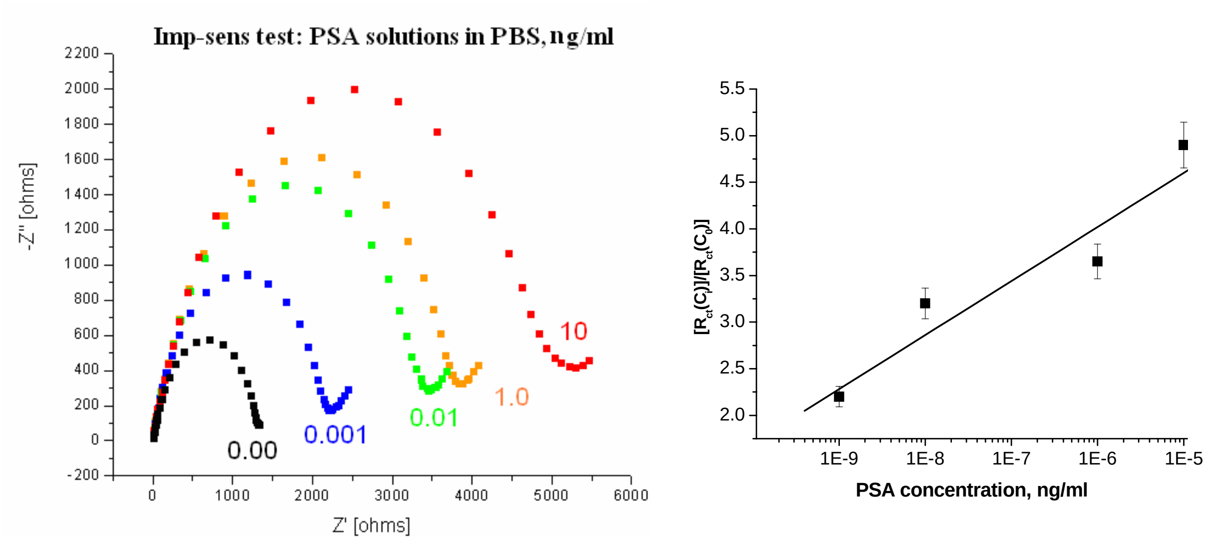
Human serum/plasma samples, prospectively collected by Dr. Phelan's lab at Moffitt Cancer Center and participating clinics, were used in this study. These samples were obtained from African-American males, either positive for (cases), or having no clinical evidence (controls) of, PCa. There was no record of other cancers available for these patients. Informed consent was obtained from all subjects at time of blood draw. In the present work, researchers were not aware of any personal identifiers, associated with the human samples.

Samples were labeled, aliquoted and stored at deep freezer (-80^0 C) until further usage according to the Moffitt Cancer Center guidelines [41]. All procedures with the samples were completed within the 30 minutes since the blood draw. No sample have undergone more than 2 freeze-thaw cycles. All samples were tested for PSA and the values were recorded.

3.5.7. Imp-sens test with the PSA solutions in PBS

Imp-sens was initially utilized to detect PSA molecules in PBS in the concentration range 1pg/nl – 10 ng/ml (Fig. 3a). This range was chosen empirically, based on the interest for meeting both current needs in PSA sensitivity (usually up to 10 ng/ml in clinics) and establishing the lower detection threshold. For each concentration, the bio-electrode was incubated in PSA solution for 30 minutes, followed by PBS washing and EIS spectra recording using PBS (10 mM, pH 7.4) containing 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe. From Fig 3a, it is clear that R_{ct} (diameter of the Nyquist plots) increases with increasing PSA concentration. The increase in R_{ct} is attributed to the binding of PSA to immobilized anti-PSA on bio-electrode, producing a packed layer that decreases the electron transfer for redox probe. For sensing application relative change in EIS data have more significant information than absolute value. Graph between change in R_{ct} values and the logarithm of PSA concentrations reveals linear detection range for PSA concentrations in the range of 1pg/ml to 10 ng/ml (Figure 3B).

Though the impedance of all electrodes fabricated in the same batch can be expected to be the same, lack of industrial scale process control in the lab leads to variations in impedance of individual electrode and antibody modified electrode. In order to confirm that observed change in impedance was due to surface modification and not due to superimposed effects, all data was normalized. For linear range detection, normalization was achieved by plotting [charge transfer resistance for desired concentration (R_{ct} (C_i))]/[charge transfer resistance of blank EA/Mab/DTSP/IDμE bio-electrode(R_{ct} (C_o))] versus logarithm of cortisol concentration (Fig 3b). After normalization, all electrodes with different impedance for bio-electrode with attached antibody exhibited similar response within the 4% error for each concentration. Fig 3b shows normalized data curve that can be characterized using $R_{ct}(C_i)/R_{ct}(C_o) = 7.50 + 0.579 \log C_{PSA}$ (g/ml). It reveals the linear range of 1pg/ml to 10 ng/ml with the correlation coefficient of 0.959. Further, to account for the variation in initial impedance values for individual electrodes, all experiments were carried out using a step-by-step approach to increasing PSA concentration. Similar step-by-step concentration studies have been reported by other researchers and help to avoid superimposed effects of multi electrode measurement [42-45].



A B

Figure 3. Imp-sens initial test with PSA, diluted in PBS. A: EIS spectra; B. Normalized curve for data obtained from EIS studies for different PSA concentrations.

As is seen from the Figure 3B, the correlation coefficient for the Imp-sens calibration curve is 0.959, suggesting a relatively weak linear trend. This effect was attributed to the interfering effects of NSB which is a common obstacle in all bioassays, employing immunological affinity principles. The NSB interference effect did not significantly change with introducing different blocking techniques (data not shown), including the use of extra strength blocking buffers and increased blocking times. Surprisingly, NSB issues were better controlled when real human serum/plasma sample was used.

3.5.8. Human plasma testing with Imp-sens

In the effort to better address the NSB issues, the real plasma sample with the lowest PSA concentration of 0.4 ng/ml, diluted 1000 times with PBS, was used as a diluent (we will further refer to this as simply a "diluent"). It is initially hypothesized that a human plasma sample, even when substantially diluted, will have enough proteins to block the initial electrode and minimize subsequent NSB during bioassays. At the same time, PSA concentration was kept low and equal which should not have interfered with the PSA detection. Using diluted plasma sample as a diluent and blocker allowed to mask major NSB issues and shift to actual complex human samples without the loss in sensitivity and selectivity.

The diluent was subsequently spiked with different PSA concentrations. 1; 10 and 100 pg/ml of PSA were chosen as initial testing points, based on the previous results (Figure 3). Providing that PSA concentration in base solution was 0.4pg/ml, the actual PSA values in the samples were 0.4; 1.4; 10.4; and 100.4; pg/ml. "Zero" or "blank" electrode impedance reading was obtained after the incubation with anti-PSA.

As mentioned earlier, all samples were tested on same electrode using the low to high concentration testing order. Similar studies were performed by other researchers and are convenient for the real time sensing. The results are shown on Figure 4. There is a clear dependence of the impedance resistance with increasing PSA concentration, indicating the PSA binding. Each step was resumed with the intense PBS washing to ensure complete removal of non-attached molecules.

An overall testing procedure schematic with Imp-sens, used in the experiment, was the following: 1. SAM formation on gold electrode (section # 2.4); 2. Anti-PSA incubation + wash + impedance reading (sections # 2.4 and 3.1); 3. Base solution ("blocker", 0.4 pg/ml PSA) incubation + wash + reading (section # 3.2.3); 4. 1 pg/ml incubation + wash + reading; 5. 10 pg/ml incubation + wash + reading; 6. 100 pg/ml incubation + wash + reading.

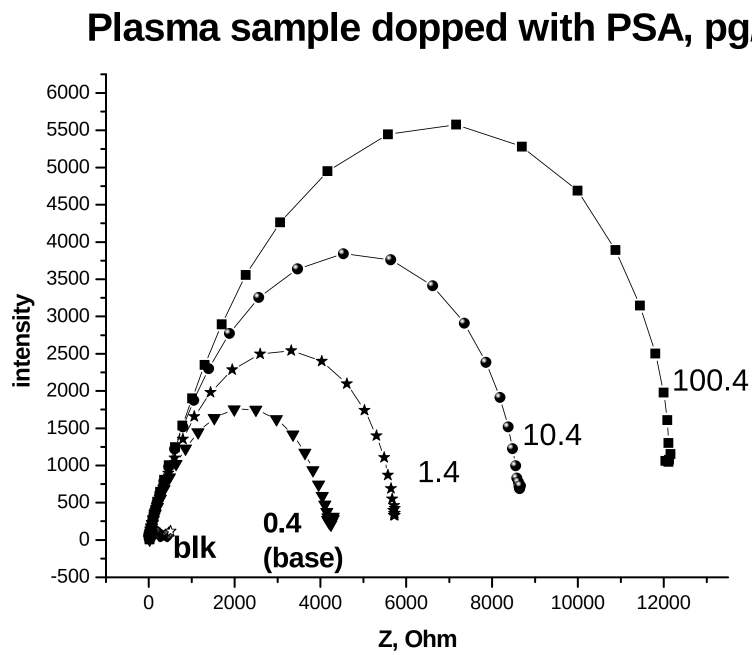


Figure 4. PSA sensing by impedance technique. BLK = electrode incubated with capture PSA antibody.

Figure 5 presents the calibration (dilution) curve, obtained with the same results. Base (0.4 pg/ml) solution reading is shown instead and is taken as a zero. As indicated, there is a strong linear concentration dependence of the impedance increase in resistance with increased PSA concentration. The correlation coefficient for this experiment was 0.995 which is an indicator of a strong linear dependence. Fig 5 shows normalized data curve that can be characterized using $R_{ct}(C_i)/R_{ct}(C_o) = 5.50 + 3.458 \log C_{PSA}$ (g/ml).

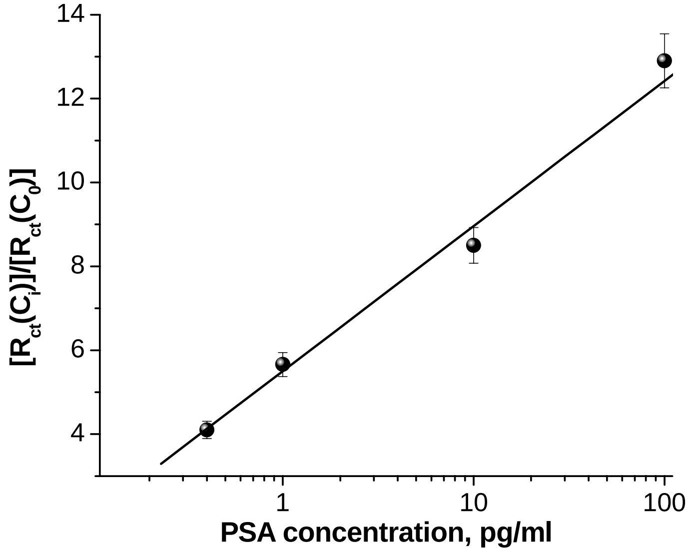


Figure 5. Normalized curve for data obtained from EIS studies for different PSA concentrations. 1000 times diluted human plasma sample was used as a blocker and a diluent to control NSB.

3.5.9. Selective binding studies

Selectivity is a major and well-known pitfall in the biosensing techniques, based on the immunological principles. To ensure the selectivity of PSA binding, the cortisol antibody with increasing concentrations in PBS were tested on the electrodes, covered with capture anti-PSA. Anti-cortisol was used as a random protein which may contribute to nonselective binding. 0; 1; 10; 100 and 1000 pg/ml of anti-cortisol in PBS was used. The testing was done in exactly same conditions. The results are shown on Figure 6. There is a slight increase of resistance with increasing anti-cortisol concentrations, however, its intensity is very low, compared to PSA (15.5% highest increase compared to 312% highest increase for PSA), indicating that the NSB is in fact present, but is negligibly small. Further assay biochemistry optimizations may lower it even more.

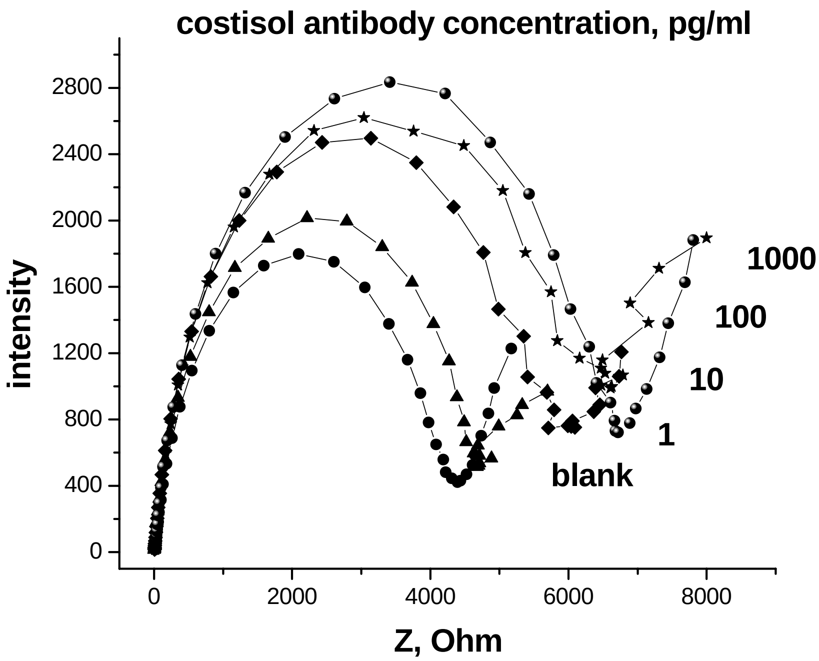


Figure 6. The interference studies of Imp-sens. Anti-cortisol binding on the bio-electrodes covered with anti-PSA.

3.6. Summary: Task 3 completed

3.7. Significance: Imp-sens, a novel portable impedimetric electrochemical immuno-sensor, can be used for an ultrasensitive and selective PSA detection. Imp-sens exhibits linear behavior in the concentration range 1pg/ml to 100 pg/ml, has low detection limit of 1 pg/ml which is at least 10 times lower than a standard commercially available, most sensitive PSA ELISA kit in the market. An average analysis time was 45 mins with Imp-sens. The sensor was found selective against cortisol antibodies in the concentrations 1 – 1000 pg/ml and was tested as a proof of concept with the human plasma sample, spiked with increasing PSA concentrations.

The main goal of this work was to establish a feasibility of using an Imp-sens as an inexpensive, reliable sensing device, suitable for point-of-care applications capable of working with complex bodily fluids like saliva, serum/plasma or urine. The sensor was therefore tested with a handful of samples and statistical analysis was out of the scope of this work. Achieving statistically significant validation of Imp-sens with a number of human serum/plasma samples is being the author's next task.

In Future, Imp-sens concept will be further expanded to validate and characterize the sensor for PSA detection with statistically significant amount of samples, as well as utilizing it for detection of other potential PCa biomarkers.

Task 4. To derive optimal cutpoints for the sensitivity and specificity of the QD-conjugated biomarkers developed in Aims 2 to 3 individually, and in combination, for the early detection of prostate cancer in African-American men. (Months 26-36 according to Timeline). Approach: Assay the levels of the individual biomarkers in serum samples collected during PSA/DRE screening using a nested case- control approach. We will use logistic regression and Receiver Operator Curves to define the cutpoints that collectively define the optimal performance of the entire panel.

4.1. Summary: See above. Our sample size is rather small for detailed statistical analysis.

C. Key research accomplishments

1. Study recruitment.
2. Established database including demographic, health, risk factor and psychosocial data.
3. Established tissue collection including multiple aliquots of plasma and sera per patient and pellet for DNA extraction.
4. Exposure in the press regarding the study and press conference at the AACR meeting. The press coverage continues and our study was highlighted in the Department of Defense annual report.
5. Achieved funding for a further year of study using aptamer technology and microfluidic devices.
6. Determined the most reproducible nanotechnology is nanowire-integrated microfluidic devices.
7. Determined that all the chosen biomarkers except PSA are not suitable biomarkers so novel biomarkers must be identified.

D. Reportable outcomes

D.1. Press Release

Invited to attend a press conference in Disparities Research at the AACR annual 2008 meeting and the study was highlighted in the Department of Defense Prostate Cancer Research Program Annual Report and other journals and health magazines.

D.2. Publications:

1. Raman scattering and SEM study of bio-conjugated core-shell CdSe/ZnS quantum dots. Phys Stat. Solid. 2: 241-243, 2007.
2. Photoluminescence spectroscopy of bioconjugated CdSe/ZnS quantum dots. Appl. Phys. Lett. 90, 263112-115. (2007). M. Dybiec, G. Chornokur, and S. Ostapenko, A. Wolcott and J. Z. Zhang A. Zajac, C. Phelan, and T. Sellers.
3. Ganna Chornokur, Sergei Ostapenko, Yusuf Emirov, Nadezhda Korsunska, Abraham Wolcott, Jin Zhang, Catherine Phelan, Abhilasha Nagaram, Thomas Sellers. Biologically Engineered Quantum Dots for Biomedical Applications// MRS Proceedings Volume 1095E**,** 1095-EE08-05, 2008.
4. G Chornokur, S Ostapenko, Yu Emirov, N E Korsunska, T Sellers and C Phelan. Spectroscopic behavior of bioconjugated quantum dots// Semicond. Sci. Technol. **23** (2008).
5. G. Chornokur, S. Ostapenko, E. Oleynik, C. Phelan, N. Korsunska, T. Kryshtab, J. Zhang, A. Wolcott and T. Sellers. Scanning Photoluminescent Spectroscopy of Bioconjugated Quantum Dots // Superlattices and Microstructures In press, 2010.
6. Ganna Chornokur, Sunil Arya, Richard Tanner, Catherine Phelan and Shekhar Bhansali . Impedance-based Miniaturized Biosensor for Ultrasensitive and Fast Prostate Specific Antigen detection: a proof of concept. In preparation, 2010.

D.3. Oral presentations:

1. ‘Early detection of prostate cancer in African-American men.’ FPCN Brother-to-Brother meetings, Municipal library, Martin Luther King Blvd, Tampa. September 2006- Catherine Phelan
2. ‘Prostate cancer in African-American men: Serum biomarkers for early detection using nanoparticles.’ Moffitt Cancer Center Genitourinary Tumor Board. October 2006. Catherine Phelan.
3. ‘Early detection of prostate cancer in African-American men.’ FPCN Brother-to-Brother meetings, Municipal

library, Martin Luther King Blvd, Tampa. March 2007 - Catherine Phelan

4. 'Prostate cancer in African-American men: Serum biomarkers for early detection using nanoparticles.' Moffitt Cancer Center Genitourinary Tumor Board. June 2007 – Catherine Phelan.
5. 'Nanoparticle-Conjugated Biomarkers for Early Detection of Prostate Cancer in African-American Men'. ImPaCT Invited speaker Sept. 2007 - Catherine Phelan.
6. 'Nanoparticle-conjugated biomarkers for early detection of prostate cancer in African-American men.' Grand Rounds in Population Sciences, Moffitt Cancer Center: October 25th 2007 - Catherine M. Phelan
7. 'Photoluminescence Spectroscopy of Bio-conjugated CdSe/ZnS Quantum Dots' Nanotech 2007 - Sergei Ostapenko.
8. 'Biomarkers' to Cancer Biology PhD students, Moffitt Cancer Center, November 8th, 2007 - Catherine Phelan.
9. 'Metal nanostructures and SERS applications', WSA Meeting, Alisomar, CA, Jan. 2007 - Jin Zhang.
10. 'Au nanoshells and nanotubes' MRS, April, San Francisco, CA, Invited – Jin Zhang.
11. 'Optical properties and applications of nanomaterials'. Second Mexican Workshop on Nanostructured Materials" during 16-18 May, invited – Jin Zhang.
12. 'Optical and dynamic properties and emerging applications of nanomaterials', International Workshop on Nanotechnology, Los Lagos, Mexico, July 2007, invited plenary – Jin Zhang.
13. 'Molecular Fiber SERS sensors for chemical and biochemical detection', ACS Western Regional Meeting, Oct., San Diego, CA, invited – Jin Zhang.
14. 'Optical properties of nanomaterials for chemical and biochemical detection applications' Singapore International Chemistry Conference 5 (SICC-5), Singapore, Dec., invited keynote – Jin Zhang
15. February. 2008, 'Early detection of prostate cancer in African-American men.' FPCN Brother-to-Brother meetings, Municipal library, Martin Luther King Blvd, Tampa - Presenter Dr. Catherine Phelan
16. March 28-29th 2008, Gastrointestinal and Genitourinary Current Therapies and Clinical Trials Agenda 'Early detection of prostate cancer in African American men.' - Presenter Dr. Catherine Phelan
17. June 2008, BIAMS symposium, Toledo, Spain. The talk title: Scanning Photoluminescent Spectroscopy of Bioconjugated Quantum Dots. Presenter Dr. Ostapenko
18. August 7, 2008, Florida A&M University Graduate/ Ph.D. Student's Schedule. Presenter Richard Tanner, MSc
19. October 30, 2008, U. S. Department of State International Visitor Leadership Program, Moffitt Cancer Center. Presenter Dr. Catherine Phelan
20. November 2008 'Biomarkers' to Cancer Biology PhD students, Moffitt Cancer Center. Presenter Dr. Catherine Phelan
21. October 2009 'Biomarkers' to Cancer Biology PhD students, Moffitt Cancer Center. Presenter Dr. Catherine Phelan

<u>D.4. Abstracts and Poster presentations</u>

1. 'Spatially Resolved Photoluminescence Spectroscopy of Quantum Dots' at the Nanoparticles Conference in Orlando, Florida (http://www.nanoparticles.org/Particles2006/)
2. 'Photoluminescence Spectroscopy of Bio-conjugated CdSe/ZnS Quantum Dots'. M. Dybiec, A. Chornokur, J. Zhang, S. Ostapenko T. Sellers, C. Phelan Nanotech 2007 meeting, June 26-28, 2007, Paris, France.
3. http://www.upperside.fr/cancernano2007/nanocancer2007program.htm
4. 'Nanoparticle-Conjugated Biomarkers for Early Detection of Prostate Cancer in African-American Men'.
5. Catherine M. Phelan, Thomas Sellers, Julio Pow-Sang, Alecsander Zajac, Abhilasha Nagaram, Jennifer Muller, Tatyana Zhukov, Raoul Salup, Sergei Ostapenko, Maciej Dybiec, Anna Chornokur and Jin Zhang. Congressionally Directed Medical Research Programs Prostate Cancer Research Program 2007 IMPaCT Meeting, Atlanta, Georgia, September 5-8, 2007.
6. 'Biologically Engineered Quantum Dots for Biomedical Applications.'G. Chornokur, S. Ostapenko, Yu. Emirov, A. Wolcott, J. Zhang, A. Nagaram and T. Sellers, C. Phelan. Abstract submitted Nov. 1 to Materials Research Society meeting (San Francisco, March 2008)
7. 'Nanotechnologies and early detection of prostate cancer'. American Association of Cancer Research, San Diego, April 2008 – abstract submitted Nov. 2007.

8. March 2008, Materi als Research Soci ety Spring meeti ng, San -Francisco, CA. The poster title: ‘ Biologically Engineered Quantum Dots for Bio medical Applications.’ G. Chor nokur, S. Ostapenko, Yu. Em irov, A. Wolcott, J. Zhang, A. Nagaram and T. Sellers, C. Phelan.
9. April 2008, ‘Nanotechnologies and early detection of prostate cancer’. American Association of Cancer Research, San Diego, April 2008. Catherine M. Phelan, Sergei Ostapenko, Anna Chornokur, Ri chard Tanner, Jin Zhang, Julio Pow-Sang, John Jack Steel, Thomas Sellers.
10. September 2008, 1st Annual Nanoscience Sy mposium "N anoFlorida 2008", Orla ndo, FL. The pos ter title: Bioconjugated QDs for early cancer detection. G. Chorno kur, S. Ostapenko, Yu. Em irov, A. Wolcott, J. Zhang, A. Nagaram and T. Sellers, C. Phelan.

<u>D.5. Degrees obtained/contributed to by this study</u>

1. <u>Maciej Dybiec</u>
‘Spatially Resolved Photoluminescence Spectroscopy of Quantum Dots’. A dissertation submitted in fulfillment of the requirements for the degree of Doctor o f Philosophy, Department of Electrical Engineering, College of Engineering, University of South Florida, Completed June 2007. Main mentor: Sergei Ostapenko.

2. <u>Ganna Chornokur</u>:
‘Photoluminescence Spectroscopy of Bio-Conjugated Quantu m Dots’, Degree of Doctor of Philosophy, Department of Electrical Engineering, College of Engineering, University of South Florida - Completed Feb 2009. Main mentor: Sergei Ostapenko; Thesis committee member: Catherine Phelan

3. <u>Marcus Marley</u>:
‘Microfluidic devices and their applic ation to early can cer detection’ . Masters degree - Co mpleted March 2008. Department of Electrical Engineering, College of Engineer ing, University of South Flori da. Main mentor: William Lee; Thesis committee member: Catherine Phelan

<u>D.5. Tissue or serum repositories</u> – We have collected DNA, serum and plasma on all cases and controls. The specimens are stored in minus 80C freezers in the Tissue Procurement Facility at the Moffitt Cancer Center. Aliquots are retrieved on request. See Appendix 1.

<u>D.6. Databases</u> – Access database from the que stionnaire and pathological data and p yschosocial aspects to risk perception. Below is based on a sub-portion of the total number of cases and controls.

				Decision making, page 13, top									
		%risk as perceived by patient		I decide		I decide, doc opinion matters		Both decide		Doc decides, my opinion matters		Doc decides	
	N	Mean	Std	N	ColPctN	N	ColPctN	N	ColPctN	N	ColPctN	N	ColPctN
All patients	110	29.74	33.20	19	100.00	35	100.00	42	100.00	11	100.00	3	100.00
marital													
	1	50.00	.	.	.	.	.	1	2.38	.	.	.	.
A)married/cohabit	72	26.12	31.45	10	52.63	27	77.14	25	59.52	8	72.73	2	66.67
B)not married/cohabit	37	35.23	36.11	9	47.37	8	22.86	16	38.10	3	27.27	1	33.33
DM_Educ													
<6th grade	1	.	.	.	.	.	.	1	2.38	.	.	.	.
6-8	2	0.00	0.00	.	.	1	2.86	1	2.38	.	.	.	.
9-10	6	12.50	25.00	3	15.79	1	2.86	2	4.76	.	.	.	.
11-12	25	18.56	16.76	.	.	6	17.14	14	33.33	4	36.36	1	33.33
GED or equivalent	13	37.70	40.30	2	10.53	2	5.71	7	16.67	1	9.09	1	33.33

	N	%risk as perceived by patient		Decision making, page 13, top									
				I decide		I decide, doc opinion matters		Both decide		Doc decides, my opinion matters		Doc decides	
	N	Mean	Std	N	ColPctN	N	ColPctN	N	ColPctN	N	ColPctN	N	ColPctN
Vocational school	30	31.63	37.95	3	15.79	15	42.86	8	19.05	3	27.27	1	33.33
Some college	22	27.12	25.37	9	47.37	4	11.43	6	14.29	3	27.27	.	.
Graduated college	10	49.38	40.22	2	10.53	5	14.29	3	7.14	.	.	.	.
Post grad or prof. school	1	100.00	.	.	.	1	2.86	.	.	.	.	.	.
Has a DRE been recommended?													
No	21	31.80	30.56	3	15.79	4	11.43	9	21.43	3	27.27	2	66.67
yes	89	29.46	33.74	16	84.21	31	88.57	33	78.57	8	72.73	1	33.33
Has a PSA been recommended?													
.	1	50.00	.	.	.	.	.	1	2.38	.	.	.	.
no	14	52.83	40.38	2	10.53	3	8.57	6	14.29	3	27.27	.	.
yes	86	28.10	33.30	17	89.47	30	85.71	29	69.05	7	63.64	3	100.00
Don't know	9	23.00	21.21	.	.	2	5.71	6	14.29	1	9.09	.	.
Do you have BPH													
no	70	28.54	30.40	12	63.16	17	48.57	29	69.05	9	81.82	3	100.00
yes	38	32.07	38.26	7	36.84	17	48.57	12	28.57	2	18.18	.	.
Don't know	2	25.00	35.36	.	.	1	2.86	1	2.38	.	.	.	.
Have you had a biopsy													
no	68	32.33	30.11	9	47.37	17	48.57	30	71.43	10	90.91	2	66.67
yes	42	25.91	37.47	10	52.63	18	51.43	12	28.57	1	9.09	1	33.33
Have you had prostatitis													
no	100	27.76	31.78	14	73.68	31	88.57	41	97.62	11	100.00	3	100.00
yes	10	44.00	41.15	5	26.32	4	11.43	1	2.38	.	.	.	.
History of cancer													
no	101	26.97	30.28	17	89.47	31	88.57	40	95.24	10	90.91	3	100.00
yes	9	52.22	47.64	2	10.53	4	11.43	2	4.76	1	9.09	.	.
Family history of cancer													
no	61	24.44	29.71	9	47.37	17	48.57	28	66.67	5	45.45	2	66.67
yes	47	39.26	36.92	9	47.37	17	48.57	14	33.33	6	54.55	1	33.33
Don't know	2	0.00	.	1	5.26	1	2.86	.	.	.	.	.	.
Family history of female cancer													
no	84	27.22	30.80	12	63.16	25	71.43	37	88.10	7	63.64	3	100.00
yes	24	41.00	40.35	6	31.58	9	25.71	5	11.90	4	36.36	.	.
Don't know	2	0.00	.	1	5.26	1	2.86	.	.	.	.	.	.

		Decision making, page 13, top											
		%risk as perceived by patient		I decide		I decide, doc opinion matters		Both decide		Doc decides, my opinion matters		Doc decides	
	N	Mean	Std	N	ColPctN	N	ColPctN	N	ColPctN	N	ColPctN	N	ColPctN
Family history breast cancer													
no	101	29.89	32.66	17	89.47	32	91.43	39	92.86	10	90.91	3	100.00
yes	9	28.14	41.40	2	10.53	3	8.57	3	7.14	1	9.09	.	.
Family history male cancer													
no	77	25.31	31.10	13	68.42	21	60.00	34	80.95	7	63.64	2	66.67
yes	31	44.75	36.00	5	26.32	13	37.14	8	19.05	4	36.36	1	33.33
Don't know	2	0.00	.	1	5.26	1	2.86	.	.	.	.	.	.
Family history prostate cancer													
no	97	27.86	32.78	15	78.95	29	82.86	41	97.62	9	81.82	3	100.00
yes	13	45.00	34.55	4	21.05	6	17.14	1	2.38	2	18.18	.	.
Psa Value													
	14	27.09	23.72	2	10.53	2	5.71	8	19.05	2	18.18	.	.
A)<=4	77	33.05	34.96	13	68.42	25	71.43	29	69.05	7	63.64	3	100.00
B)>4	19	18.36	31.21	4	21.05	8	22.86	5	11.90	2	18.18	.	.
Number of PSA in 5 years													
	29	41.06	33.06	2	10.53	8	22.86	13	30.95	5	45.45	1	33.33
A)<=5	71	25.65	32.43	15	78.95	23	65.71	26	61.90	5	45.45	2	66.67
B)>5	10	31.50	36.06	2	10.53	4	11.43	3	7.14	1	9.09	.	.
Number of DRE in 5 years													
	20	37.71	32.47	4	21.05	6	17.14	7	16.67	1	9.09	2	66.67
A)<=4	58	28.42	33.89	5	26.32	19	54.29	27	64.29	6	54.55	1	33.33
B)>4	32	27.48	32.99	10	52.63	10	28.57	8	19.05	4	36.36	.	.

D.7. Funding applied for based on work supported by this award

1. Florida Nanotechnology Center of Excellence in Early Detection of Cancer (Sellers PI)
2. Nanotechnology Sensors and Systems for Early Detection of Cancer (Sellers PI)
3. Lustgarten foundation – 'Enhanced detection of pancreatic cancer using advanced molecular probes (Phelan PI).
4. Cancer Research and Prevention Foundation – 'Quantum dot-conjugated biomarkers for early detection of breast cancer in Mexico (Phelan PI)
5. Florida Nanotechnology Center of Excellence in Early Detection of Cancer (Sellers PI) (2006)
6. Nanotechnology Sensors and Systems for Early Detection of Cancer (Sellers PI) (Dec. 2007) ('Microfluidic devices for early detection of breast cancer in Mexico (Phelan PI of pilot study))
7. Lustgarten foundation – 'Enhanced detection of pancreatic cancer using advanced molecular probes (Phelan PI) (2007)
8. NIH R21 'Biologically Engineered Quantum Dots for Biomedical Applications.' PI Sergei Ostapenko (Not funded)
9. NIGMS R25 training grant for funding for minority predoctoral students. PI Bernard Batson.

10. NSF 'The Application of Quantum Dots and Nanowires in Early Cancer Detection.' PI Sergei Ostapenko (Not funded)
11. NIH Health Disparities Center of Excellence P20. 'Aptamer-based microfluidic devices for early prostate cancer in African American men.' RO1 format PI – Catherine Phelan. Not Funded.
12. Moffitt Cancer Center/University of Florida Joint Cancer Center Funding Opportunity Advancing the Partnership (AP) Awards. PI Catherine Phelan – Funded to July 2010 - $99,931

E. Conclusion

Implications of completed research: We have investigated emerging cutting-edge nanotechnologies, for early detection of prostate cancer in African-American men. Despite our initial enthusiasm for QDs as the nanotechnology of choice, we determined that QD technology is cost-prohibitive for use in a large population-based screening and lacks reproducibility. We investigated another type of nanotechnology - nanowire-integrated microfluidic devices and found these to function well for sub-ELISA level detection and will employ this technology in the future. Initially we chose six candidate biomarkers but showed they are unusable either due to technical difficulties in our hands or their levels did not show differences between cases and controls or they decreased in concentration through increasing stage of disease. PSA is still the most optimal biomarker for prostate cancer detection. So our final research (manuscript in preparation) brings these two components together for the most optimal prostate cancer detection.
The samples collected in this study are essential for other studies in African-American men. We are planning to continue recruitment by expanding the collection Florida state-wide. Furthermore we are collaborating with other researchers in a consortium known as 'Men of African Descent with Cancer of the Prostate -MADCaP' for future studies.

"so what section" – The novel, sensitive biomarkers generated in this study will be of vital importance for early detection of prostate cancer in African-American men to reduce the morbidity and mortality from this disease. We are applying novel technologies. Furthermore, we are applying our knowledge and these technologies for early detection of other cancer types.

F. References

1. Warren C, Chan W and Nie S 1998 Quantum dot bioconjugates for ultrasensitive nonisotopic detection *Science* 281 2016-18.
2. Human PSA ELISA Kit from Abazyme: http://www.biocompare.com/itemdetails.asp?itemid=434262
3. JURINCIC-WINKLER C. ; VON DER KAMMER H. ; HORLBECK R. ; KLIPPEL K.-F.; PIXBERG H. U. ; SCHEIT K.-H. ; Clinical evaluation of a new prostate-specific antigen sandwich ELISA which employs four monoclonal antibodies directed at different epitopes of prostate-specific antigen. European Urology 1993, vol. 24, n4, pp. 487-491
4. Dybiec M, Chornokur G, Ostapenko S, Wolcott A, Zhang JZ, Zajac A, Phelan C and Sellers T 2007 Photoluminescence spectroscopy of bioconjugated CdSe/ZnS quantum Dots *Appl. Phys Lett* 90 263112
5. G Chornokur, S Ostapenko, Yu Emirov, N E Korsunska, T Sellers and C Phelan Spectroscopic behavior of bioconjugated quantum dots // Semicond. Sci. Technol. 23 (2008) 075045.
6. Ganna Chornokur, Sergei Ostapenko, Yusuf Emirov, Nadezhda Korsunska, Abraham Wolcott, Jin Zhang, Catherine Phelan, Abhilasha Nagaram, Thomas Sellers. Biologically Engineered Quantum Dots for Biomedical Applications// MRS Proceedings Volume 1095E**,** 1095-EE08-05, 2008.
7. Raman scattering and SEM study of bio-conjugated core-shell CdSe/ZnS quantum dots. Phys Stat. Solid. 2: 241-243, 2007.
8. Diaz Cano A, Torchynska TV, Dybiec M, Ostapenko S, Jiménez-Sandoval S, Vorobiev Y, Phelan CM, Zajac A, Zhukov T Raman scattering and SEM study of bio-conjugated core-shell CdSe/ZnS quantum dots. *Physica Status Solidi,* 4: 241-243, 2007.
9. Dybiec, M., "Spatially Resolved Photoluminescence Spectroscopy of Quantum Dots" PhD Theses, USF, 2006.
10. N. S. Ramgir, A. Zajac, P. K. Sekhar, L. Lee, T. A. Zhukov, S. Bhansali; "Voltammetric Detection of Cancer Biomarkers Exemplified by Interleukin-10 and Osteopontin with Silica Nanowires"; J. Phys. Chem. C 2007, 111, 13981-13987
11. S. Aravamudhan, A. Kumar, S. Mohapatra, S. Bhansali, Sensitive estimation of total cholesterol in blood using Au nanowires based micro-fluidic platform, Biosens. Bioelectron. 22 (2007) 2289– 2294.
12. A. Kumar, S. Aravamudhan, M. Gordic, S. Bhansali, S.S. Mohapatra, Ultrasensitive detection of cortisol with enzyme fragment complementation technology using functionalized nanowire, Biosens. Bioelectron. 22 (2007) 2138–2144.
13. R. A. Rahman, C-M. Lo, S. Bhansali, "A MEMS Micro-Electrode Array Bio-sensor for Impedance Spectroscopy of Human Umbilical Vein Endothelial Cells", Sensors & Actuators B 118, 115-120, 2006.
14. N. S. Ramgir, A. Zajac, P. K. Sekhar, L. Lee, T. Zhukov and S. Bhansali, Ultrasensitive Voltammetric Detection of IL-10, a Lung Cancer Biomarker, in Serum using SiO_2 Nanowires Template", Sensors Letters, in press (2008).
15. S. Aravamudhan, A. Kumar, S. Mohapatra, S. Bhansali "Sensitive Estimation of Total Cholesterol in Blood using Au Nanowires based Micro-Fluidic Platform," Biosensors & Bioelectronics 2008 (in press)..
16. Kumar, S. Aravamudhan, M. Gordic, S. Bhansali, S. Mohapatra, "Ultra sensitive detection of cortisol with enzyme fragment complementation technology using functionalized nanowires," Biosensors & Bioelectronics, 2008 (in press).
17. C. Dhand, S. K. Arya, M. Datta and B. D. Malhotra, *Anal. Biochem*., 2008, 383, 194–199.
18. A. Kumar, S. Aravamudhan, M. Gordic , S. Bhansali and S. S. Mohapatra, *Biosens. Bioelectron*., 2007, 22, 2138–2144.
19. S. M. Radke and E.C. Alocilja, *Biosens. Bioelectron*., 2005, 20, 1662–1667.
20. E. Bakkar and Y. Qin, *Anal. Chem*., 2006, 78, 3965–3984.
21. S. Alegret, Integrated Analytical Systems, vol. XXXIX, Elsevier B.V., Amsterdam, 2003 (Chapter 1).
22. S. K. Arya, P. R. Solanki, R. P. Singh, M. K. Pandey, M. Datta and B. D. Malhotra, *Talanta,* 2006, **69**, 918–926.
23. K. Oka, M. Noguchi, T. Kitamura and S. Shima, *Clin. Chem*., 1987, 33, 1639–1642.
24. D. Appel, R. D. Schmid, C. A. Dragan, M. Bureik and V. B. Urlacher, *Anal. Bioanal. Chem*., 2005, 383, 182–186.
25. R. Gatti, E. Cappellin, B. Zecchin, G. Antonelli, P. Spinella, F. Mantero and E. F. De Palo, *J. Chromatography B*, 2005, 824, 51–56.
26. I. Jerkunica, J. Sophianopoulos and D. Sgoutas, *Clin. Chem*., 1980, 26, 1734-1737.
27. M. Vogeser and P. Mojmle, J. Briegel, *Clin. Chem. Lab. Med*., 2007, 45, 521-525.
28. N. J. Cook, A. L. Schaefer, P. Lepage and S. D. M. Jones, *J. Agric. Food Chem*., 1997, 45, 395-399

29. D. Schmaldng, W. Nashabeh, X. W. Yao, R. Mhatre, F. E. Regnier, N. B. Afeyan and M. Fuchs, *Anal. Chem.*, 1996, 67, 606-612.
30. W. A. Kaptein, J. J. Zwaagstra, K. Venema, M. H. J. Ruiters and J. Korf, *Sens. Actuators B*, 1997, 45, 63–69.
31. M. Sarkar, B. C. Das, B. D. Bora, V. Kumar, K. Mohan, H.H.D. Meyer and B. S. Prakash, *Gen. Comp. Endo.*, 2007, 154, 85–90.
32. J. S. Mitchell, T. E. Lowe and J. R. Ingram, *Analyst*, 2009, 134, 380–386
33. A. J. Bard, M. Stratmann and P. R. Unwin, Instrumentation and Electroanalytical Chemistry, vol. 3, Wiley-VCH, New Jersey, 2003 (Chapters 1–2).
34. E. Barsoukov and J. R. MacDonald, Impedance Spectroscopy: Theory, Experiment and Applications, second ed., Wiley Interscience, New Jersey, 2005 (Chapter 1).
35. K. Sun, N. Ramgir, S. Bhansali, *Sens. Actuators B*, 2008, 133, 533–537.
36. E. Katz and I. Willner, *Electroanalysis*, 2003, 15, 913–947.
37. J. Guan, Y. Miao and Q. Zhang, *J. Biosci. Bioeng.*, 2004, 97, 219–226.
38. A.W. Bott, *Curr. Sep.* 2001, 19, 71–75.
39. Moffitt Cancer Center and Research Institute Official Website: www.moffitt.org
40. R. C. Stevens, S. D. Soelberg, S. Near and C. E. Furlong, *Anal. Chem.* 2008, 80, 6747–6751.
41. R. Maalouf, C. F. Wirth, J. Coste, H. Chebib, Y. Saikali, O. Vittori, A. Errachid, J. P. Cloarec, C. Martelet and N. J. Renault, *Anal. Chem.*, 2007, 79, 4879-4886.
42. X. L. Su and Y. Li, *Biosens. Bioelectron.*, 2004, 19, 563–574.
43. Y. Huang, M. C. Bell and I. I. Suni, *Anal. Chem.*, 2008, 80, 9157–9161.
44. Y. G. Lee and K. S. Chang, *Talanta*, 2005, 65, 1335–1342.
45. T. T. N. Lien, T. D. Lam, V. T. H. An, T. V. Hoang, D. T. Quang, D. Q. Khieu, T. Tsukahara, Y. H. Lee and J. S. Kim, *Talanta*, 2010, 80, 1164-1169.

Example of number of aliquots of plasma, sera and DNA

Patient ID #	TCC from Moffitt	0=control, 1=case	Location of patient	Date of collection	Status at date of recruitment	Histology	PSA	# of Plasma aliquots	# of Serum aliquots	DNA total amount/ volume*
#001	yes	1	Moffitt	01/14/2008	being treated with seed implants	T2a prostate cancer, 3+3 Gleason 6, and biopsy. In 04/2007, he had seed implants.	8.4 on 12/2006, 3.1 on 12/2007	5	4/1.0 ml &1/0.75 ml	not extracted from pellet
#002	yes	1	Moffitt	02/28/2008	just diagnosed	T2a NX MX Gleason 6 PC in 6 out of 12 biopsies diagnosed in 07/2007. Treatment for PC deferred because of radio/chemo for squamous cell carcinoma neck mass to finish in 03/2008.	2.2 on 12/2007	3/1.9 ml	2/2.0 ml & 1/1.0 ml	not extracted from pellet
#004	yes	1	Moffitt	03/24/2008	PC	T1C N0 M0 prostate cancer, Gleason 7. Biopsy on 12/11/2007	6.7 on 12/20/2007	4/1.0ml	3/1.1ml	not extracted from pellet
#005	yes	1	Moffitt	03/26/2008	PC	biopsy performed on 02/2008 revealed prostate cancer and a Gleason 3+3 in 1 out of 12 cores compromising 8% of that core.	4.4 on 2/06/2008	2/1.5 ml&1/1.0 ml	2/1.5 ml	not extracted from pellet
#006	yes	1	Moffitt	04/02/2008	PC	T1C NX MX, Gleason 7, ultrasound on 3/04/2008	4.1 on 2/06/2008	5/1.0ml	4/1.0ml	not extracted from pellet
#007	no	0	Moffitt	04/04/2008	control at LCS	Normal	2.2 on 4/04/2008	3/1.7	2/1.7 &1/0.75	not extracted from pellet
#008	yes	0	Moffitt	04/16/2008	just diagnosed	patient had prostatectomy on 1/08/2008. Diagnosed on 7/02/2007 with 3+3 in 1 of 5 cores, 5% all tissue.	undectable on 4/04/2008, 3.3 on 7/02/2007	2/2 ml & 1-1 ml	2/2 ml	not extracted from pellet
#009		1	Tampa	05/15/2008	PC	gleason 3+3 =6 two of 2 cores One core 35% the other core had 40% cancer	7.6 on 4/15/2008	1/1ml	2/2ml & 1/1ml	not extracted from pellet
#010		1	Tampa	05/23/2008	PC	gleason 3+3=6 one of two cores positive one core had 10% cancer	5.5 on 4/16/2008	2/1.7ml	2/1.7 &1/0.75	not extracted from pellet
#011	no	1	Moffitt	05/28/2008	PC	Diffuse Gleason 8 cancer involving 90% of his specimen on right side and 75% of his specimen on left side on 4/15/2008.	79 on 4/16/2008	3/1.7	3/1.7	not extracted from pellet
#12	no	1	Moffitt	05/28/2008	PC	1c Nx Mx Gleason 6 and 7 on 4/22/08	5.08 on 3/2008	3/1.7	2/1.7	not extracted from pellet
#13	yes	1	Moffitt	06/04/2008	PC	T1c N0 M0, Gleason score 8, 3 scores out of 8 on 3/11/2008	21, 3/08/2008	3/1.7	2/1.7	not extracted from pellet
#014	yes	1	Moffitt	06/11/2008	PC	T1c NX MX, Gleason 6, 5 out of 6 biopsies on 5/09/2008	4.7, 6/08/2008	3/1.1.5	3/1.5	not extracted from pellet
#015		1	Tampa	06/20/2008	PC	TIC, NX, MP, Stage II	6.9 on 3/31/2008	2/2 & 1/0.5ml	1/2 & 1/1ml	not extracted from pellet
#016		1	Tampa	06/20/2008	PC, previous lymphoma	T1C, WX, MO, stage II, 3 + 3 in 1 out of 3 on 5/2008	6.9 on 03/17/2008; 4.8 on 6/11/08	3/2ml	1/2ml	not extracted from pellet
#017	yes	1	Moffitt	06/25/2008	PC	Gleason 3 + 4 in 5 out of 12 cores at 10-70%	5.7, on 2/2008	2/1.7 ml&1/1.2 ml	2/1.5 ml	not extracted from pellet
#018	yes	1	Moffitt	07/02/2008	PC	T1c Nx M0 Gleason 6 prostate cancer in 2 out of 12 biopsies	4.6 on 5/2008	3/1.7ml	2/1.7 ml	not extracted from pellet
#019	yes	1	Moffitt	07/09/2008	PC	Gleason 10; 8 out of 12 with Gleason 9; and 1 out of 12 with Gleason 8. All of the cores except for the Gleason 8 were high volume of 60%-90% involvement. The Gleason 8 core was 10% involvement.	67 on 4/2008	3/1.5ml	3/1.5ml	not extracted from pellet
#020		1	Tampa	07/15/2008	PC	4 + 3 on 70% of cores.	4.98 on 5/2008	3/1.7ml	2/1.7 & 1/0.75ml	not extracted from pellet
#021		1	Brandon	07/16/2008	PC	3 + 3 on13% of cores on 5/13/2008	10.9 on 3/13/2008	2/1.8 ml	2/2.0 ml	not extracted from pellet
#022	no	0	Moffitt	07/18/2008	control at LCS	mild enlarged prostate on 7/18/2008	3.1 on 7/18/2008	2/2.0 ml&1/0.5 ml	2/2.0 ml	not extracted from pellet
#023	no	0	Moffitt	08/01/2008	control at LCS	asymetric, right>left on3/2008	2.0 on 3/2008	1/1.5ml	1/1.5ml	not extracted from pellet
#024	yes	0	Moffitt	08/06/2008	control at GU	BPH, 51cc, biopsy negative	1.3 on 8/2008	3/1.7ml	3/1.4ml	not extracted from pellet
#025	yes	1	Moffitt	08/06/2008	PC	T1C NO MO Gleason 8 on right side on 8/27/2008	4.2 in 07/2008	2/1.75 ml	2/1.9 ml	not extracted from pellet
#026		1	Tampa	08/15/2008	PC	T1C NX MO II on right 2%	10.00 on 5/2008	3/1.5 ml	2/1.25 ml	not extracted from pellet
#027	yes	1	Moffitt	09/03/2008	PC	T2, NO, MOP, Gleason 7 (3+4) on the left lateral base, left lateral mid, left80%, 60%, and 40% respectively. Right side biopsies negative, 12 biopsies on 7/1/08	4.5 on 7/2008	3/1.1ml	3/1.0ml	not extracted from pellet

Example of number of aliquots of plasma, sera and DNA

Patient ID #	TCC from Moffitt	0=control, 1=case	Location of patient	Date of collection	Status at date of recruitment	Histology	PSA	# of Plasma aliquots	# of Serum aliquots	DNA total amount/ volume*
#028		1	Tampa	09/04/2008	PC	3+3 on 2 of 7 cores with 40-80% on 4/12/2006	18 on 08/2008	2/1.8 ml	2/1.8 ml	not extracted from pellet
#029	yes	0	Moffitt	10/01/2008	just diagnosed	patient had a prostatectomy on 5/14/2007. His gleason was 3+3 at time of surgery with a PSA of 0.4	.01 on 4/2008	2/1.8 ml	2/1.8 ml	not extracted from pellet
#030	yes	1	Moffitt	10/15/2008	PC	3+3=6 on 2 of 10 cores in <5% on 9/4/2008	3.7 on 10/2008	2/1.7, 1/0.5 ml	2/1.7 ml	not extracted from pellet
#031	yes	1	Moffitt	10/22/2008	PC	T1c NX MX Gleason 8, 4+4, 3 out of 12 cores in the right mid apex 65%,right lateral apex 30%, right lateral mid 30%.	4.6 on 5/2008	2/1.8 ml	2/1.7, 1/0.5 ml	not extracted from pellet
#032		0	30th Street	10/23/2008	control	N/A		4/1.0ml	2/1.0ml	not extracted from pellet
#033		0	30th Street	10/23/2008	control	Visited Dr. Sterling (GU). DRE on 10/31/2008. 2+ prostate, smooth, history of BPH	3.3 on 10/25/2008, 4.2 on 04/10/2006	2/1.0 & 1/0.5ml	2/1.0ml	not extracted from pellet
#034		0	30th Street	10/28/2008	control	N/A	0.8 on 12/27/2008	2/1.5, 1/1.0 ml	2/1.5 ml	not extracted from pellet
#035	yes	1	Moffitt	10/29/2008	PC	biopsy on 10/06/2008, 3+4 on 6 cores, left apex with 60% and left with 25%	09/08/2008 at 2.62	3/1.5 ml	2/1.7 ml	not extracted from pellet
#036		0	30th Street	10/30/2008	control	N/A	2.1 10/29/2008	2/1.8, 1/1.5 ml	2/1.8	not extracted from pellet
#037	yes	1	Moffitt	11/03/2008	PC	gleason 6 on right lateral mid with less than 5%	4.4 on 02/2008	2/1.5 ml	2/1.2ml	not extracted from pellet
#038		1	Tampa	11/18/2008	PC	T1C NX MO II with 30%, 5% on right	10.8 on 11/17/2008	3/1.3 ml	2/1.4ml	not extracted from pellet
#039		0	30th Street	11/18/2008	control	N/A	4.1 on 5/15/2008, 2.0 on 4/02/2007	2/2.0, 1/1.0ml	1/2.0, 1/0.75ml	not extracted from pellet
#040		0	30th Street	11/18/2008	control		N/A	2/2.0ml	1/2.0ml	not extracted from pellet
#041	yes	1	Moffitt	11/19/2008	PC	T1C, NX, MO, 3+3=6, <%5, 6 of 12 on right, on 7/21/2008	5/30/2008 at 4.3	1/0.7ml	0	not extracted from pellet
#042	no	1	Moffitt	11/19/2008	PC	T1C NX MO, 3+6 in 1 out of twelve at 3%.	3.7 on 08/2008	2/1.6ml	2/1.5ml	not extracted from pellet
#043		0	30th Street	11/20/2008	control	N/A	1.2 on 10/15/2008	2/2.0ml	1/2.0,1/1.5ml	not extracted from pellet
#044		0	30th Street	11/20/2008	control	N/A	0.8 on 1/14/2008	2/2.0,1/1.0ml	1/2.0,1/1.0ml	not extracted from pellet
#045		1	Tampa	11/24/2008	PC	3+3 T2a NX MO Stage II on 4/15/2008	4.0 on 08/12/2008	3/1.8 ml	2/1.7 ml	not extracted from pellet
#046		0	30th Street	12/02/2008	control	N/A	0.5 on 08/29/2008	2/2.0 & 1/1.5ml	2/2.0ml	not extracted from pellet
#047		1	30th Street	12/04/2008	PC		1.8 on 11/24/2008	2/2.0 & 1/0.5ml		not extracted from pellet
#048		0	30th Street	12/09/2008	control	N/A	1.1 on 9/22/2008	2/2.0 & 0.75ml	1/2.0,1/0.5ml	not extracted from pellet
#049		0	30th Street	12/09/2008	control	N/A	0.7 on 05/2007	3/1.5 ml	1/1.0ml	not extracted from pellet
#050		0	30th Street	12/09/2008	control	N/A	1.1 on 1/13/2009	2/2.0 & 1/1.5ml	2/1.8ml	not extracted from pellet
#051		0	30th Street	12/10/2008	control	N/A	1.5 on 7/23/2008	2/2.0ml	2/2.0 & 1/1.0ml	not extracted from pellet
#052		0	30th Street	12/16/2008	control	N/A	0.7 on 11/2007	2/2.0 & 1/1.0 ml	2/2.0ml	not extracted from pellet
#053		0	30th Street	12/16/2008	control	N/A	3.9 on 08/09/2007	2/2.0ml	1/250ul	not extracted from pellet
#054		0	30th Street	12/16/2008	control	N/A	1.1 on 06/08/2008	1/2.0 ml & 1/1.25 ml	1/600ul	not extracted from pellet
#055		0	30th Street	12/30/2008	control	N/A	0.4 on 12/12/2006	2/2ml &1/250ul	1/1.8 ml	not extracted from pellet
#056		0	30th Street	01/08/2009	control	N/A	1.0 on 1/14/2009	1/2 ml & 1/1.2 ml	1/1.8ml&1/2.0ml	not extracted from pellet

Example of number of aliquots of plasma, sera and DNA

Patient ID #	TCC from Moffitt	0=control, 1=case	Location of patient	Date of collection	Status at date of recruitment	Histology	PSA	# of Plasma aliquots	# of Serum aliquots	DNA total amount/ volume*
#057		0	30th Street	01/13/2008	control	N/A	0.57 on 08/19/2008	2/2ml	1/1.6 ml	not extracted from pellet
#058		0	30th Street	01/15/2009	control	N/A	0.3 on 10/13/2008	3/1.3ml	1/2 & 1/0.5ml	not extracted from pellet
#059		0	30th Street	01/22/2009	control	N/A	4.2 on 1/17/2008	2/1.5ml	2/1.4ml	not extracted from pellet
#060		0	30th Street	01/22/2009	control	N/A	0.4 on 2/14/2008	2/1.9ml	1/1.8ml	not extracted from pellet
#061		0	30th Street	01/22/2009	control	N/A	1.4 on 1/26/2009	2/1.8ml	2/1.5ml	not extracted from pellet
#062		0	30th Street	01/27/2009	control	N/A	0.5 on 5/12/2008	2/1.8ml	2/1.6ml	not extracted from pellet
#063		0	30th Street	01/27/2009	control	N/A	0.4 on 8/13/2008	3/1.4ml	2/1.3ml	not extracted from pellet
#064		0	30th Street	01/27/2009	control	N/A	1.3 on 12/31/2008	2/1.2ml	2/1.3ml	not extracted from pellet
#065	yes	1	Moffitt	01/28/2009	PC	T1C, NX, MX, 3+3=6, 1 of 12 cores on right on 10/28/2008	5.6 on 10/2008	2/1.1ml	2/1.2ml	not extracted from pellet
#066		0	30th Street	01/29/2009	control	N/A	2.4 on 8/21/2008	1/1.0 ml	3/1.5ml	not extracted from pellet
#067		1	Brandon	02/05/2009	PC	T1C, NX, MO, II, on 12/12/2008	4.0 on 12/2008	1/2.0 ml & 1/1.8 ml	0	not extracted from pellet
#068		1	Brandon	02/05/2008	PC	T1C, NX, MO, II, 3+3 on 8 of 12 cores on1/16/2009	4.1 on 10/2008	2/1.6ml	1/2.0 ml & 1/1.0 ml	not extracted from pellet
#069		0	30th Street	02/10/2009	control	N/A	0.6 on 10/2008		2/1.3ml	not extracted from pellet
#070		1	Brandon	02/11/2009	PC					